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IONIZING RADIATION IN THE PRODUCTION OF BACTERIAL PREPARATIONS

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IONIZING RADIATION IN THE PRODUCTION OF BACTERIAL PREPARATIONS

- USSR -

/Following is the translation of an article by V.I. Troitskiy in the Russian-language publication Meditsinskaya Radiologiya (Medical Radiology), Vol 2, No 5, 1957, pages 80-88./

From the Institute of Epidemiology and Microbiology imeni N. F. Gamaley, Academy of Medical Sciences USSR.

This article presents experimental data obtained in the Department of Medical Microbiology of the Institute imeni N. F. Gamaley by M. A. Tumanyan, Z. G. Pershina, V. M. Vadimov, D. R. Kaulen, I. M. Goncharenko, A. P. Duplishcheva, and T. S. Sedova, with the participation of V. G. Khrushchev.

The foreign literature contains developed suggestions on cold sterilization of antibiotics, but except for general expressions on the possibility of using ionizing radiation in the production of medical bacterial preparations, we have not found specific suggestions in this area. The production of bacterial preparations is an important branch of the medical industry. We refer to the production not only of medicinals, such as serums having naturally limited application, but also of prophylactics, such as preventive vaccines, which have been given to millions of persons.

Various approaches are possible for the use of ionizing radiation in producing bacterial preparations. Above all we have in mind the use of ionizing radiation for cold sterilization. In the production of bacterial preparations sterilization is the main necessary element of any technological process. It is carried out either in autoclaves with superheated steam under pressure, or by high temperature in dry-heat chambers, or finally by the addition of antiseptics.

The possibility of sterilizing preparations hermetically packed or poured into ampules has given rise to a sterilization method that is more improved and reliable than those presently existing. On the other hand, in the use of radiation sterilization in producing bacterial preparations qualitative change of the preparation is possible, the harmful effects induced by other sterilization methods, for example, the use of formalin or heat employed to sterilize vaccines, can be eliminated. It also must be kept in mind that sterilization through autoclaving has found widest application in bacteriological institutes. Therefore, the steam approach

is an inseparable part of bacteriological production. Introducing the cold sterilization method would free institutes producing serums and vaccines from the constant need to be supplied with steam production.

As objects of sterilization in the bacteriological industry as well as in bacteriological research work are the following: nutrient media for raising bacteria, killed microbial vaccines, the so-called "chemical" vaccines -- antigenic complexes extracted from microbial bodies, anatoxins, therapeutic serums, glass dishes widely used in bacteriological work, and finally, the production wastes of the bacteriological industry which must be rendered harmless, for which purpose several large autoclaves are in daily use in large institutes.

A prerequisite for developing the use of radiational sterilization in all the areas referred to is the determination of the bactericidal dose of the γ-rays, deadly not only to the vegetative forms of bacteria but also to spore-borne microbes. In the methods of cold sterilization of bandaging materials and penicillin that have been suggested by the American investigators a sterilizing dose of 1,500,000-2,000,000 r has been indicated.

In our experiments even in the sterilization of soil samples, including top-dressing soils containing particularly resistant spore-borne forms of microbes it has been found that irradiation in a dose of 1,500,000 r assures the sterility of the sample irradiated. A lesser dose -- 1,000,000 r -- kills the overwhelming majority of such microbes. However, about 0.01% of the microbes, evidently the most radioresistant, remain viable. In addition, the experiments have shown that to kill intestinal group bacteria even at a bacteria concentration in suspension of 20 billion to 30 billion microbial bodies per 1 ml a dose of 600,000 r is sufficient. The photographs presented, taken with an electric microscope (Figure 1), show that the bactericidal action of large doses of γ-rays is related to destruction of the microbial cells, their lysis, which however does not eliminate their immunogenic properties.

Under the irradiation conditions we employed we settled on a dose of 1,500,000 r as providing complete sterilization of the irradiated objects. The radiation source in our experiments was an experimental γ-irradiator (EGO-2), which consists of a set of radioactive cobalts (Co^{60}) preparations with a total activity of 5000 C (80,000 r-eq. Ra). The dose strength was 600 r/min. Irradiation was conducted in a field uniform as to volume with a deviation not greater than 5%. Below are presented several experimental data substantiating the means of using ionizing radiation in producing bacterial preparations. We carried out a study on those biological and immunological indices which are primarily important for evaluating our preparations.

Figure 1. Electronic microphotographs: effect of γ-rays on B. coli. Magnification: 6400.

1, 2 -- unirradiated; 3-6 -- irradiated; 3 -- at a dose of 50,000 r; 4 -- at a dose of 300,000 r; 5, 6 -- at a dose of 1,000,000 r.

Use of Ionizing Radiation for Sterilizing Nutrient Media

One of the most widespread nurient media in medical bacteriology is the Hauttinger agar, the basis of which consists of the products of tryptinic digestion of meat. A prepared medium was divided into three parts: one was subjected to usual sterilization in an autoclave at 120°, another, following autoclaving, was irradiated with a dose of 1,500,000 r, while the third was sterilized by irradiation at 1,500,000 r.

An unpleasant putrid odor, which gradually disappeared over the next several days, was noted in the irradiated media. The media was tested 1, 7, and 14 days following sterilization. Cultures were inoculated into media poured into Petri dishes. Two strains of typhoid fever bacteria and two strains of Flexner dysentery bacteria

were tested. For the inoculation the same amount of each of the bacterial suspensions was used. The number of colonies growing in the dishes after a 24-hour incubation in a thermostat at 37° C was determined.

As seen from Table 1, where the number of colonies growing in the medium sterilized by autoclaving is taken as 100%, in all the experiments in which the media were tested at various times following sterilization the number of colonies growing in the irradiated media always exceeded the number of colonies growing in the autoclave-sterilized medium. In several cases this number was very substantial (180-200%).

Table 1

Growth of Bacteria in Irradiated Media

(a)	(b) %	(c) 1-e сутки		(d) 7-е сутки		(e) 14-е сутки	
		(f)	(g)	(f)	(g)	(f)	(g)
B. typhi 2		144,9	141,2	405,8	116,5	131	113
B. typhi 4446		147	134	192,5	126,5	110	138,5
B. Flexner 28		202,8	138,8	134,4	181,8	110,5	133,8
B. Flexner 170		138,8	147,5	109	122,9	101	131

LEGEND: a) Species of bacteria; b) % of growing colonies; compared to the autoclaved medium; c) 1st day; d) 7th day; e) 14th day; f) Autoclaved and irradiated media; g) Irradiated medium.

Thus, irradiation not only does not decrease the nutrient properties of meat media for intestinal group bacteria, but even to a certain extent enhances these properties. This can perhaps be explained by the absence in radiation sterilization of the destructive action of high temperature on several growth factors, which in autoclave sterilization can impair the nutrient properties of the medium. However, as seen from the data presented the media first autoclaved and then irradiated also exceeded in nutrient properties the media sterilized only by autoclaving. More probable is the suggestion that as a result of proteolysis of the protein particles as yet uncleaved, the amount of amino acid requisite for growth and propagation of bacteria rises.

It was also important to find out whether irradiation of the broth impedes the formation therein of diphtheria toxin, which is the starting product for preparation of diphtheria anatoxin. The

experiments showed that in media irradiated even with a dose of 1,500,000 r, no less and usually even more toxin is formed than in media sterilized in the autoclave.

Effect of Ionizing Radiation on the Antigenic and Immunogenic Properties of Bacteria

Only sparse data exist on the effect ionizing radiation has on antigenic and immunogenic properties of bacteria. Nevler has studied the action of radon on these properties.

In our experiments we studied the immunogenic properties of typhoid fever and Flexner dysentery bacteria killed by irradiation at a dose of 1,500,000 r (radio-vaccines) or killed by formalin, and then irradiated, as well as studying antigenic complexes extracted from microbial bodies killed by ionizing radiation, or antigenic complexes obtained from formalinized microbial bodies which had subsequently been irradiated at a dose of 1,500,000 r.

In our experiments the immunogenic properties were evaluated according to the resistivity index determined by testing the immunity of mice or rats vaccinated with the given preparation. Here, we took as the resistivity index the ratio of the LD_{50} of a live culture for the immunized animals to the LD_{50} of a live culture for the control non-immunized animals. In other experiments evaluation of immunogenic properties was based on the percentage of mice surviving following infection of the animals by the corresponding culture in an absolutely fatal dose. The experimental results are given in Table 2.

As seen from the data presented in Table 2, we can state that no substantial difference exists in the immunogenic properties of corpuscular vaccines and those of antigenic complexes obtained by killing bacteria with formalin or ionizing radiation. It must be noted that for typhoid fever bacteria following their treatment with a dose of 1,500,000 r the Vi-antigen is preserved, as this is shown by the reaction of hemagglutination with anti-Vi serum, and the ability to induce antibody formation upon immunization of animals is also wholly preserved. The experiments also have shown that there is no substantial difference either in the dynamics or in the titer of the antibodies formed as a result of immunization by radio-vaccines or formalin vaccines (Table 3).

It is also important that irradiation does not increase the toxicity to laboratory animals of the typhoid fever and dysentery vaccines killed by ionizing radiation.

Thus, an approach has been uncovered for using ionizing radiation in the preparation of intestinal vaccines of both the corpuscular and the chemical types.

Table 2

Immunogenic Properties of Radio-Vaccines

(a) Препарат	(b) Количество животных	(c) 50 млн. микробных тел	(d) Индекс резистентности	(e) % выживших
(h) Формоловакцина B. typhi	40 мышей (f)	274	5,9	
(i) То же, облученная в дозе 1 700 000 r	37 »	212	4,2	
(j) Формоловакцина B. Flexner 4437	40 »	1080	3,0	
(k) Радиовакцина B. Flexner 4437	40 »	1000	2,9	
(l) Антиген из формалинизированной культуры B. typhi	49 крыс (g)	3200	16	
(m) То же, облученный в дозе 1 500 000 r	50 »	2400	12	
(n) Антиген из культуры B. typhi, облученной в дозе 1 500 000 r	50 »	2500	12,8	
(o) Антиген из формалинизированной культуры B. Flexner 170	48 »	—	—	33,4
(p) То же, облученный в дозе 1 500 000 r	47 »	—	—	38,0
(q) Антиген из культуры B. Flexner, облученной в дозе 1 500 000 r	46 »	—	—	48,0

LEGEND: a) Preparation; b) No of animals; c) 50 million microbial bodies; d) Resistivity index; e) % surviving; f) mice; g) rats; h) Formalin vaccine of B. typhi; i) Same, irradiated with a dose of 1,700,000 r; j) Formalin vaccine of B. Flexner 4437; k) Radio-vaccine of B. Flexner 4437; l) Antigen from formalinized culture of B. typhi; m) Same, irradiated with a dose of 1,500,000 r; n) Antigen from a culture of B. typhi, irradiated with a dose of 1,500,000 r; o) Antigen from formalinized culture of B. Flexner 170; p) Same, irradiated with a dose of 1,500,000 r; q) Antigen from culture of B. Flexner irradiated with a dose of 1,500,000 r.

Table 3

Antigenic Properties of Serums From Rabbits Immunized by Radio-Vaccines

(a) Иммунизация	(b) Средние титры агглютининов				
	I	II	III	IV	V
(c) Радиовакцина B. typhi	450	4100	5630	5120	1280
(d) Формолвакцина " "	1280	5380	5380	6140	1280
(e) Радиовакцина Flexner 26	900	1150	3580	1380	1150
(f) Формолвакцина "	450	770	1080	1152	830

Symbols: I -- on the 7th day following the 1st immunization;
II -- on the 7th day following the 2nd immunization;
III -- on the 7th day following 3rd immunization;
IV -- on the 14th day following the 3rd immunization;
V -- on the 21st day following the 3rd immunization.

LEGEND: a) Immunization; b) Mean titer of agglutinis; c) Radio-vaccine of B. typhi; d) Formalin vaccine of B. typhi; e) Radio-vaccine of Flexner 26; f) Formalin vaccine of Flexner 26.

Effect of Ionizing Radiation on Anatoxins

Diphtheria and tetanus anatoxins were subjected to irradiation at a dose of 1,500,000 and 2,000,000 r. Both native preparations as well as those adsorbed on aluminum oxide hydrate were irradiated, adsorption generally being known to increase the immunogenic properties of preparations.

To test the immunogenic properties of irradiated diphtheria anatoxin small pigs were immunized twice (with native preparations) or once (with the adsorbed preparation). The immunity was checked a month after the immunization had been completed by administering various toxin doses to the young pigs. The experimental results are presented in Table 4.

As seen from Table 4, irradiation did not change the immunogenic properties of native anatoxin. However, it greatly altered the immunogenic properties of the adsorbed anatoxin.

The immunogenic properties of irradiated tetanus anatoxin was determined by experiments on white mice. The experimental results are presented in Table 5.

Table 4

Effect of γ-rays on Diphtheria Anatoxin Irradiation with a Dose of 1,500,000 r

(a) Иммунизация свинок анатоксином	(b) Доза токсина в Dlm	(c) % выживаемости свинок, иммунизированных анатоксином	
		(d) необлучённые	(e) облучённые
(f) Нативный	30	50	54,4
	20	85,7	92,3
(g) Адсорбированный	1000	0	0
	500	22,3	0
	250	80	9,1
	125	93,4	50

LEGEND: a) Immunization of pigs with anatoxin; b) Toxin dose in Dlm; c) % survival rate of pigs immunized with anatoxin; d) non-irradiated; e) irradiated; f) native; g) adsorbed

The results in Table 5 were obtained somewhat differently from those for irradiation of diphtheria anatoxin. In spite of the fact that irradiation was carried out at a still higher dose -- 2,000,000 r, the immunogenic properties were preserved not only for the irradiated native anatoxin, but the adsorbed preparations following irradiation hardly differed in immunogenic properties from the original unirradiated anatoxin. Thus, the native anatoxins can be subjected to radiation sterilization without loss of their immunogenic properties. The problem of sterilizing adsorbed preparation calls for further research.

Effect of Ionizing Radiation on Antitoxin Serums

Both native anti-diphtheria serums as well as those concentrated and purified by the Diatherm-3 method have been subjected to radiation. The serums were irradiated with a dose of 1,500,000 r, and also with a dose of 600,000 r, which as indicated above is sufficient to kill the vegetative forms of many bacteria. The antitoxin titer was determined in vitro by the flocculation method, and also in guinea pigs by the Remer method. The relative viscosity of the serums was also determined.

As seen from Table 6, irradiation in a dose of 600,000 r either does not reduce the antitoxic titer of the serums at all or reduces it very negligibly. Irradiation at a dose of 1,500,000 r

however sharply reduces the antitoxin titer in the serum irradiated. This decrease amounted to 30% for the native serums, and for the purified -- 15-20%. An even greater decrease in antitoxin titer was observed upon irradiation with a dose of 1,500,000 r of anti-perfringens serum.

Table 5

Irradiation of Tetanus Anatoxin
Dose of 2,000,000 r

(a)	(b)	(c)	
		(d)	%
(e)			
(f)	60	14	23,3
(g)	60	18	30
(h)			
(f)	57	57	100
(g)	80	49	86

LEGEND: a) Immunization; b) Number of animals;
c) Survived; d) Absolute number;
e) Native anatoxin, two-fold immunization;
f) Nonirradiated; g) Irradiated
h) Adsorbed anatoxin, single immunization

Electrophoretograms and spectrograms of irradiated serums showed that large radiation doses induce physicochemical changes in them corresponding to the intensity of the irradiation, but precisely what reactions derive therefrom has thus far not been discovered. Figure 2 presents photographs of electrophoretograms of normal and irradiated serums, and also microphotograms taken of such serums with the photoelectric MF-2 microphotometer. From the electrophoretograms it is clear that at high doses the globulin fractions are degraded and, perhaps, new fractions are formed, but it is impossible to determine how many. The spectrograms (Figure 3) give only a picture of the intensity of the process occurring as a function of the dose of irradiation. The spectrophotometric curves of the absorption coefficients in the ultraviolet portion of the spectrum yields for the irradiated and unirradiated serum absorption maximums and minimums in the same parts of the spectrum. The curves run parallel, differing only in intensity.

Table 8

Effect of γ-rays on Anti-Diphtheria Serums

(a) Сыворотка	(b) № кролика	(c) Титр антитоксина по (g) флокуляции			(d) Ремеру			(e) Относительная вязкость		
		(f) необлученные	600 000 r	1 800 000 r	(f) необлученные	600 000 r	1 800 000 r	(f) необлученные	600 000 r	1 800 000 r
Натявая (h)	1	890	—	—	900		600	1,75	2,2	2,65
	2	730	730	—	650	600	450	2,0	2,3	2,7
	3	1210	1000	—	1200		900	1,8	2,3	2,4
	4	615	—	—	600	500	400	2,0	2,2	2,7
	5	1150	1150	—	1100		900	2,1	2,4	2,8
	6	730	730	—	650		450	1,65	2,0	2,2
Диатерм-3 (i)	7	2500	2350	2100	2500	2400	2000	2,7	2,7	3,0
	8	2500	2500	2000	2500	2400	2000	3,2	3,4	3,7
	9	2100	2100	2000	2100	2100	1800	4,3	4,5	4,8
	10	2100	2100	1900				3,6	3,8	4,0
	11	2350	2350	2100				4,0	4,3	4,5
	12	2000	2000	1810				4,8	4,1	4,5

LEGEND: a) Serums; b) Number of rabbits; c) Anatoxin titer according to; d) Remer; e) Relative viscosity; f) Nonirradiated; g) flocculation; h) Native; i) Diatherm-3

The data presented afford several preliminary conclusions on the ways of using ionizing radiation in producing bacterial preparations.

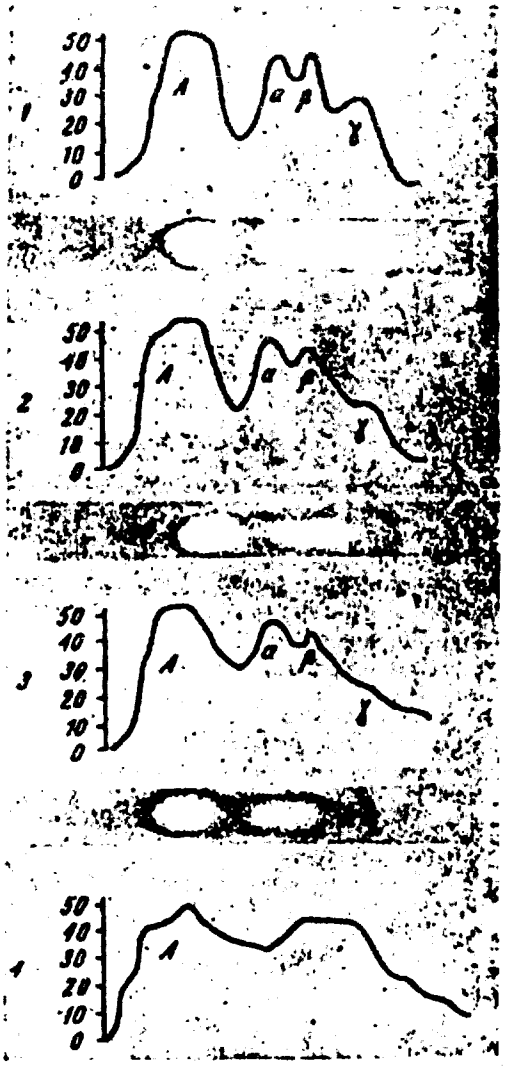

Figure 2. Electrophoretograms of irradiated serums

1 -- normal, 2-4 -- irradiation;
2 -- with a dose of 500,000 r;
3 -- with a dose of 1,000,000 r;
4 -- with a dose of 2,000,000 r;
A -- albumins; α, β, γ -- globulins

The starting point for dealing with this question is the proven position on the sterilizing effect of γ-rays. We were able to confirm this position already well known in the literature by our own investigations. Here we must say that we are dealing now with the use of the sterilizing action namely of γ-rays, since in the range of radiation energy of interest to us they do cause induced radioactivity, which would make the method of cold sterilization practically inapplicable. In addition, it also was important to be convinced through the experiments that the sterilizing doses of γ-rays act bactericidally even on thick suspensions of

microbial bodies, which are obtained by using modern methods of producing microbial vaccines, and amount to concentrations of 20 billion to 30 billion microbial bodies and more per 1 ml.

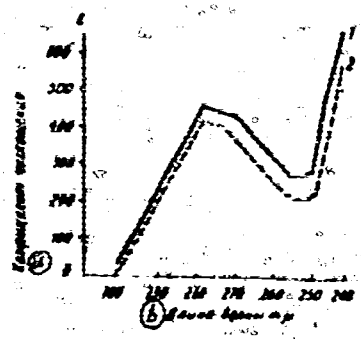

Figure 3. Spectrophotometric curves of absorption coefficients of blood serum of rabbits irradiated with X-ray

1 -- normal; 2 -- irradiated with a dose of 200,000 r.

LEGEND: a) Absorption coefficient;
b) Wavelength in mμ

The possibility of cold sterilization of bacterial production wastes -- an infectious material both in the liquid and semisolid form contaminating glassware, as well as the cold sterilization of clean glassware before placed in the production process does not give rise to any doubt. The most economical radiation equipment with sufficient passing power corresponding to the production capacity of the given institute must be developed.

As has been shown, means have been found for the use of ionizing radiation in preparing nutrient media, in producing corpuscular killed and chemical intestinal vaccines, and diphtheria and tetanus anatoxins. Still called for is a special study of cold sterilization of therapeutic serums. Here it is important to note that in several cases we are not dealing with the use of more improved and reliable sterilization methods, but of how to produce new types of preparations.

For the practical solution of the problem of ionizing radiation use in producing bacterial preparations, in addition to further study of the physical, chemical, and immunological changes in the irradiated objects, solutions must be found to the technical problems

of the radiation sources and irradiator designs. It may be supposed that the most suitable ionizing radiation source can be enriched fission products. The hypothetical value of the source is 250,000-300,000 C. Such a source would provide a passing capacity of the equipment up to 6,000-7,000 r per day (for a 20-hour working day) and a sterilization dose of 1,500,000 r. Based on technological grounds the equipment could advisably be used as a submerged radiation source. With a proper selection of source configuration and method of irradiation, the effectiveness of radiation use can reach 60-70%.

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